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Linalool reduces the expression of 3-hydroxy-3-methylglutaryl CoA reductase via sterol regulatory element binding protein-2- and ubiquitin-dependent mechanisms

Sung-Yun Cho, Hee-jin Jun, Ji Hae Lee, Yaoyao Jia, Kyoung Heon Kim, Sung-Joon Lee *

Department of Biotechnology, The Graduate School of Biotechnology, Korea University, Seoul 136-713, Republic of Korea

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ABSTRACT

We investigated hypocholesterolemic mechanisms of linalool, an aromatic anti-oxidative monoterpene, which is abundant in teas and essential oils. Oral administration of linalool to mice for 6 weeks significantly lowered total and low-density lipoprotein cholesterol concentrations, and HMG-CoA reductase protein expression (−46%; $P < 0.05$) by both transcriptional and posttranscriptional mechanisms. Linalool suppressed the gene expression of HMG-CoA reductase by reducing the binding of SREBP-2 to its promoter, as assessed by qPCR and chromatin immunoprecipitation, and by inducing ubiquitin-dependent proteolysis of the HMG-CoA reductase. These findings suggest that food molecules with a pleasant scent could exert beneficial metabolic effects through multiple mechanisms.

1. Introduction

Hypercholesterolemia is a key risk factor in atherosclerosis, with the formation of atheromas after cholesterol buildup in the coronary arteries, and the subsequent development of coronary heart disease (CHD) [1]. In a meta-analysis, a 10 mg/dl reduction in plasma cholesterol level was shown to decrease the CHD mortality by up to 9% [2]. This level of cholesterol decrease could be achieved by a nutritionally balanced diet that included a low intake of saturated fat and cholesterol and a high intake of fiber and phytochemicals [3]. Phytochemicals from herbs and teas may reduce plasma cholesterol levels, which may be important for preventing CHD, especially in those with mild hypercholesterolemia and elevated CHD risk [4].

The cholesterol-lowering activity of teas has been intensively investigated [5]. Tea derived from the leaves of *Camellia sinensis* is the world's most popular and widely consumed beverage. Studies on the hypocholesterolemic effects of teas have focused particularly on green tea catechins as key active compounds [6], yet the results regarding green tea catechins in humans have not been consistent [7], although data from a meta-analysis have suggested a significant reduction in low-density lipoprotein (LDL) cholesterol associated with green tea intake [8]. The possibility exists that other compounds in teas may contribute to plasma cholesterol reduction and that small aromatic anti-oxidative terpenes in teas may be candidates. However, metabolic functions of tea terpenoids have been largely ignored [9]. The small aromatic terpenes are also present at high concentrations in herbal essential oils. Several recent in vitro and in vivo metabolic studies on herbal essential oils rich in small aromatic terpenoids have been shown to reduce plasma cholesterol levels by downregulating 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) expression [10,11].

Linalool (MW, 154.25 g/mol) is a naturally occurring small terpene with a pleasant scent and strong anti-oxidative activity [12,13]. Linalool is a major or common compound in most herbal essential oils and teas, including both green and black teas [14]. Linalool is characterized by a pleasant floral scent and is present in more than 200 species of plants in families such as Lamiaceae (mints, scented herbs), Lauraceae (laurels, cinnamon, rosewood), and Rutaceae (citrus fruits). The strong antioxidant activity of linalool inhibits LDL oxidation, which enhances cholesterol uptake via macrophage scavenger receptors [12,13]. Therefore, this molecule may exhibit in vivo anti-atherogenic activity.

In this study, we examined the hypocholesterolemic effects of linalool in high-fat fed C57BL/6J mice and HepG2 cells. Linalool significantly decreased total and LDL cholesterol concentrations by

Abbreviations: HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; SREBP-2, sterol regulatory element binding protein-2; CHD, coronary heart disease; HFD, high-fat diet; ChIP, chromatin immunoprecipitation; 25-HC, 25-hydroxycholesterol; SRE, sterol regulatory element; CRE, cAMP responsive element; PCAF, P300/CBP-associated factor; S1P, site-1 protease; S2P, site-2 protease; LDL, low-density lipoprotein; CYP, cytochrome P450

* Corresponding author. Fax: +82 2 3290 3029.
E-mail address: junelee@korea.ac.kr (S.-J. Lee).

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reducing sterol regulatory element binding protein-2 (SREBP-2) expression and a concomitant reduction in HMGCR expression via the acceleration of ubiquitin-dependent degradation and downregulation of its transcription.

2. Materials and methods

An expanded Materials and methods section, including chemicals and reagents, plasma lipid determination, cellular lipid determination, isolation of total RNA and reverse-transcription PCR, ubiquitination of HMGCR, chromatin immunoprecipitation (ChIP) assay, and immunocytochemistry, is available in the Supplementary materials available online.

2.1. Animals

Male C57BL/6J mice (Samtako Korea, Gyeonggi-Do, Korea), 10–12 weeks old, were divided into three groups (6 mice per group, 26–30 g body weight) and fed an AIN-76A-based high-fat diet (HFD) containing 45% of the total calories from fats, 20% from proteins, and 35% from carbohydrates for 5 weeks prior to linalool administration. Then, the mice were continuously fed the same HFD during the oral administration of linalool for an additional 6 weeks. Two doses were examined in the animal experiments: low (0.57 mg/mouse/d) and high (120 mg/mouse/d) levels. Previously, we reported hypocholesterolemic effects of *Plantago asiatica* essential oil, which contained 82% (v/w) linalool [11], and the low concentration was extrapolated from the effective dose of *P. asiatica* essential oil. The high concentration was determined as the highest non-toxic concentration based on the data for cellular toxicity and LD_{50} [15,16]. The high concentration was reported as non-toxic in mice and showed anti-inflammatory activity. At 0, 3, and 6 weeks, blood samples were collected retroorbitally into EDTA-treated tubes from mice fasted overnight for 8–10 h. Plasma samples were obtained by centrifugation at 12 000×*g* for 15 min at 4 °C and stored at −20 °C until analysis. The mice were killed after the feeding period. The organs were snap-frozen in liquid nitrogen and stored at −80 °C for RNA and protein analyses. All animal procedures were performed in accordance with a protocol approved by the Animal Experimentation and Ethics Committee of Korea University (protocol No. KUIACUC-20090420-4).

2.2. Cell culture and linalool treatment

HepG2 cells were seeded in 6-well Falcon plates at 5×10^5 cells/well in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% PEST (contains 100 units/ml penicillin and 100 μg/ml streptomycin in cell culture grade water for gram-positive and gram-negative bacteria). Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2 until they were 60–80% confluent before using them for RT-PCR, Western blot analysis, ChIP assay, and immunocytochemistry. The culture medium was changed every 2 days, and the cells were kept in serum-free media during the treatment period. Linalool was dissolved in dimethyl sulfoxide (DMSO) to the final treatment concentrations. Cells were exposed to 0, 0.1, and 0.5 mM linalool for 24 h.

2.3. RNA isolation and quantitative real-time PCR (qPCR)

Total RNA was extracted from HepG2 cells and mouse livers using a TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol. For cDNA synthesis, 2 μg of total RNA was reverse-transcribed using Superscript II reverse transcriptase (Invitrogen), according to the manufacturer's protocol, and a combination of oligo(dT)15 primers, resulting in 20 μl of cDNA. PCR was performed using 1 U of Taq DNA polymerase (Promega, Madison, WI, USA) and 1 μl of cDNA template. The primers used for PCR are listed in Supplementary Table 1. Real-time PCR was performed in a reaction volume of 20 μl containing 10 μl of iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA), 1 μl of primers (10 μM), 1 μl of cDNA, and sterile water. PCR conditions were 95 °C for 5 min, followed by 50 cycles of 95 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min. A melting curve of the amplified DNA was analyzed at temperatures between 54 and 95 °C at a heating rate of 0.2 °C/s. Quantitative PCR was performed in an iCycler iQ instrument (Bio-Rad). During primer extension, the fluorescence from the amplified DNA was recorded using the SYBR Green optic channel set at a wavelength of 490 nm. Data were collected and viewed using iCycler iQ optical system software version 5 (Bio-Rad).

2.4. Immunoblotting and nuclear and membrane protein isolation

Proteins were isolated from the nuclear and membrane fractions using a kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. SDS–PAGE and immunoblotting were performed as described previously [11]. A Bio-Rad Chemidoc XRS system and software were used to capture chemiluminescent images, and band intensities were determined using the Bio-Rad Quantity One image analyzing program.

2.5. Data analysis

All experiments were repeated at least three times. Quantifiable data are shown as means ± S.E.M. The significance of differences was evaluated using Student's *t* test and the Tukey–Kramer multiple-comparison *post hoc* test.

3. Results

3.1. Linalool has hypocholesterolemic effects

We investigated the lipid-lowering ability of linalool in mice. Body weight and food intake were similar among groups during the feeding period (Supplementary Fig. 1A and B). After 6 weeks of oral administration, linalool significantly reduced plasma TG, total cholesterol, and LDL levels (Fig. 1A). In comparison with controls, total cholesterol levels were reduced by 14% in both the low (0.57 mg linalool/mouse/d) and high linalool groups (120 mg linalool/mouse/d) at 6 weeks, and triglycerides were reduced markedly by 40% and 31% at 3 and 6 weeks, respectively, in the low linalool group. LDL cholesterol levels were also significantly reduced by 45% and 42% at both 3 and 6 weeks, respectively, in the low linalool group compared with controls. At 6 weeks, HDL cholesterol tended to increase in both the low (+26%) and high (+14%) linalool groups. Several atherogenic indexes showed that the plasma lipoprotein profiles were improved after linalool administration (Table 1). The livers of control mice fed the HFD showed lipid accumulation, but this accumulation of lipids was markedly reduced in the livers of mice fed linalool at both high and low concentrations, and the overt, large lipid droplets had disappeared as assessed by immunohistochemistry after oil red O staining (data not shown). Hepatic lipid levels measured in HepG2 cells showed a dose-dependent reduction of cellular cholesterol and TG concentrations by 34% and 37%, respectively, at 1 mM linalool (Fig. 1B).

3.2. Linalool reduces SREBP-2 expression and attenuates nuclear translocation

The expression of SREBP-2, a key transcription factor in cellular cholesterol homeostasis, was quantified. Linalool-treated HepG2 cells showed a 66% ($P < 0.01$) reduction in the level of SREBP-2

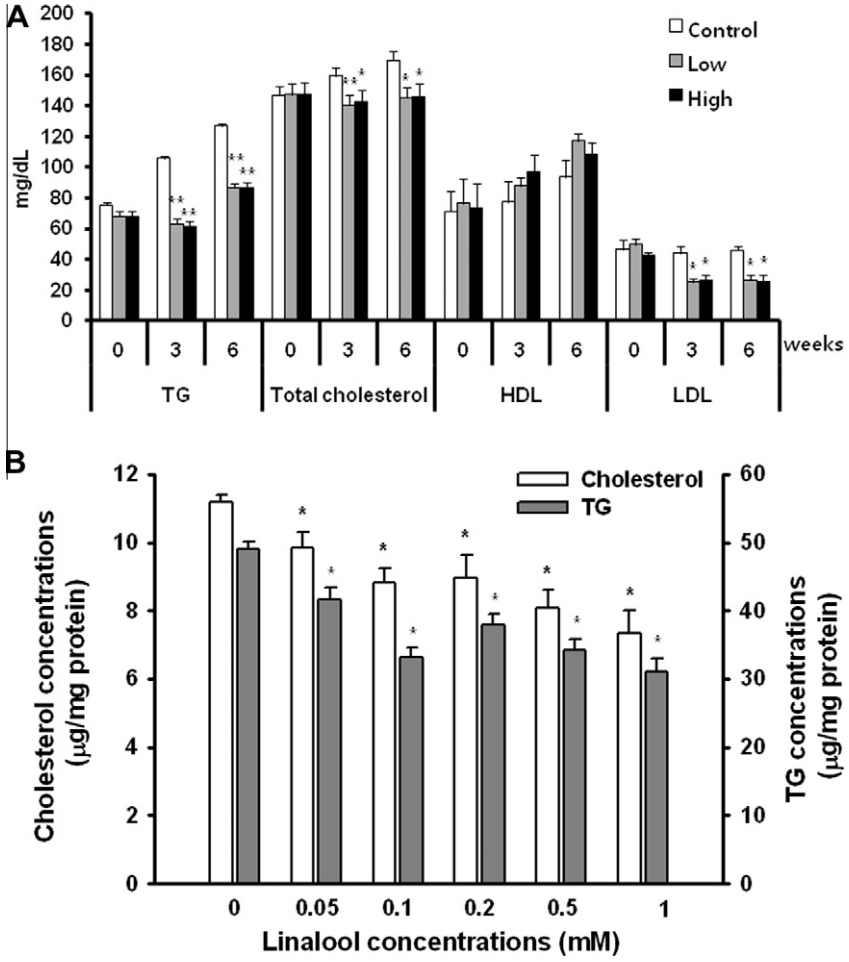

Fig. 1. Hypolipidemic activities of linalool. (A) Plasma triglyceride (TG), total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) concentrations after linalool administration in mice. Control mice were fed the same volume of water. Low (0.57 mg/mouse/d) and high doses of linalool (120 mg/mouse/d) were orally administered to mice daily for 6 weeks. (B) Cellular hepatic cholesterol and TG levels. A more detailed method is provided in the Supplementary methods. Data are presented as means ± S.E.M. (n = 3 per group). $^{*}P < 0.05$ compared with control; $^{**}P < 0.01$ compared with control.

Table 1
Effects of linalool on plasma atherogenic indices in high-fat diet-induced obese C57BL/6J mice.

Atherogenic index	Week 0	Week 3	Week 6
LDL-C/HDL-C			
Control	0.65 ± 0.05	0.65 ± 0.02	0.58 ± 0.01
Low	0.57 ± 0.04	0.29 ± 0.04**	0.27 ± 0.03**
High	0.49 ± 0.02	0.23 ± 0.07**	0.23 ± 0.06**
TG/HDL-C			
Control	1.06 ± 0.01	0.89 ± 0.02	0.92 ± 0.02
Low	1.37 ± 0.01	0.72 ± 0.06**	0.63 ± 0.03**
High	1.36 ± 0.01	0.74 ± 0.07**	0.80 ± 0.05*
TC/HDL-C			
Control	2.06 ± 0.02	1.93 ± 0.02	2.00 ± 0.02
Low	2.06 ± 0.02	1.60 ± 0.02**	1.47 ± 0.01**
High	1.82 ± 0.02	1.46 ± 0.01**	1.34 ± 0.01**

* $P < 0.05$.
** $P < 0.01$.

mRNA expression at 0.5 mM. In mouse livers, linalool at the low concentration significantly decreased SREBP-2 mRNA expression by 60% ($P < 0.01$) as well. In HepG2 cells analyzed by immunoblotting, the precursor form of SREBP-2 (pSREBP) decreased by 37% and 49% at 0.1 and 0.5 mM, respectively. The nuclear form of SREBP-2 (nSREBP) was reduced by 35% and 65% at 0.1 and 0.5 mM linalool, respectively (Fig. 2A). In mouse livers, the low linalool concentration reduced the expression of the nuclear and precursor forms of SREBP-2 by 68% and 55%, respectively. In HepG2 cells, the nuclear-to-precursor ratio of SREBP-2 protein was lowered by 32% at 0.5 mM linalool compared with controls. A similar trend was found in mouse livers since decreases of almost 30% were observed for both concentration of linalool.

We further investigated SREBP-2 expression in HepG2 cells by immunohistochemistry and confocal microscopy. In controls, SREBP-2 protein was highly expressed and evenly localized in both the cytoplasm and nucleus (Fig. 2B); however, after treatment with 0.1 mM or 0.5 mM linalool, SREBP-2 protein levels were markedly reduced in the cells, especially in cells incubated with 0.5 mM linalool. Confocal images were examined to quantify the distribution and prevalence of the SREBP-2 protein. After normalization, linalool-treated cells (0.5 mM) showed reductions in the SREBP-2 protein by 50% and 65% for the precursor and mature form, respectively, compared with controls, confirming a reduction in SREBP-2 protein with linalool treatment ($P < 0.01$; Fig. 2C). In addition, the nuclear-to-precursor ratio of SREBP-2 protein was also decreased by 30% at 0.5 mM linalool compared with controls, which was similar to the result obtained from immunoblotting analysis. These observations suggest that linalool reduced the gene expression of SREBP-2 and attenuated posttranscriptional nuclear translocation of SREBP-2.

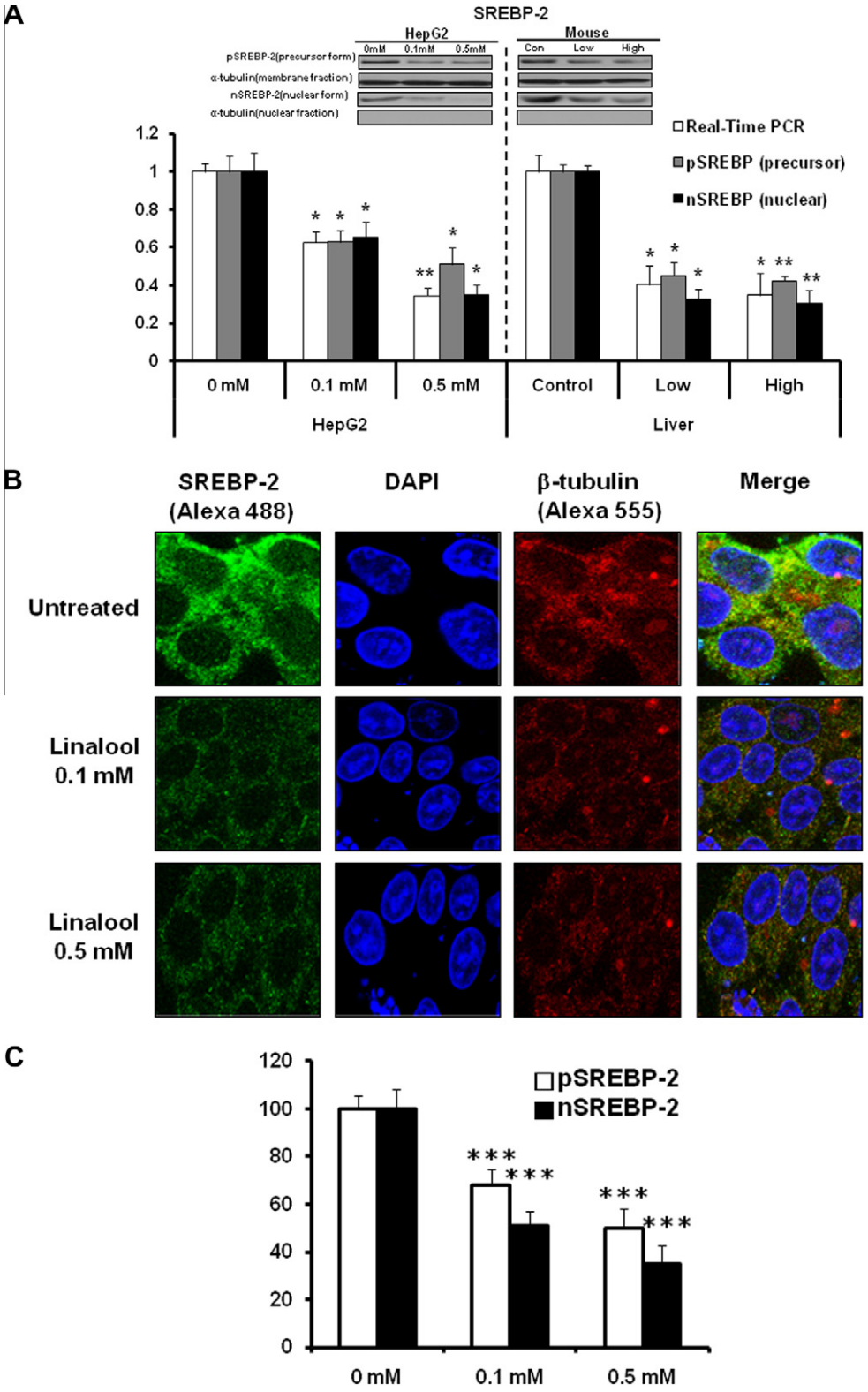

Fig. 2. Expression of SREBP-2. HepG2 cells and mouse livers after linalool treatment as described in Section 2. (A) SREBP-2 mRNA and protein expression. (B) Cellular localization of SREBP-2. The immunohistochemistry procedure is provided in the Supplementary methods. Images (×1000) were obtained by confocal microscopy. The cellular distribution and expression of SREBP-2 protein (Alexa 488, green) with respect to the nucleus (DAPI, blue) and β-tubulin (Alexa 555, red) as a housekeeping gene (red). (C) SREBP-2 protein expression was quantified by image analysis. MetaMorph 7.6 (Molecular Devices, Sunnyvale, CA, USA) was used for image analysis. Data are presented as means ± S.E.M. ($n = 3$ per group). $^{*}P < 0.05$ compared with control; $^{**}P < 0.01$ compared with control.

3.3. Expression of HMGCR and Insig-dependent ubiquitination

In both HepG2 cells and mouse livers, the levels of HMGCR mRNA expression, as determined by both RT-PCR and qRT-PCR, were reduced significantly (up to −45%) with 0.5 mM linalool (Fig. 3A). HMGCR protein expression decreased by 54% with 0.5 mM linalool in vitro. Low and high concentrations of linalool reduced HMGCR mRNA expression by 66% and 79%, respectively

($P < 0.05$) in mice as assessed by qRT-PCR. Protein levels were reduced by 54% ($P < 0.01$) at 0.5 mM linalool in vitro and 55% ($P < 0.05$) in the low linalool group compared with controls (Fig. 3A). No significant changes were found in LDL receptor or CYP7A1 mRNA expression in vitro or in vivo (Supplementary Fig. 2).

HMGCR protein is degraded by Insig-dependent ubiquitination, and oxysterols such as 25-hydroxycholesterol (25-HC) stimulate the process [17]. The expression of Insig-1 was upregulated with linalool treatment and feeding (Fig. 3B); thus we further investigated whether linalool accelerated the ubiquitination and degradation of HMGCR. Detergent-solubilized cell extracts were subjected to immunoprecipitation with polyclonal antibodies against the reductase, and the resulting immunoprecipitates were immunoblotted with an anti-ubiquitin antibody. In the anti-ubiquitin immunoblots of reductase immunoprecipitates from cells treated

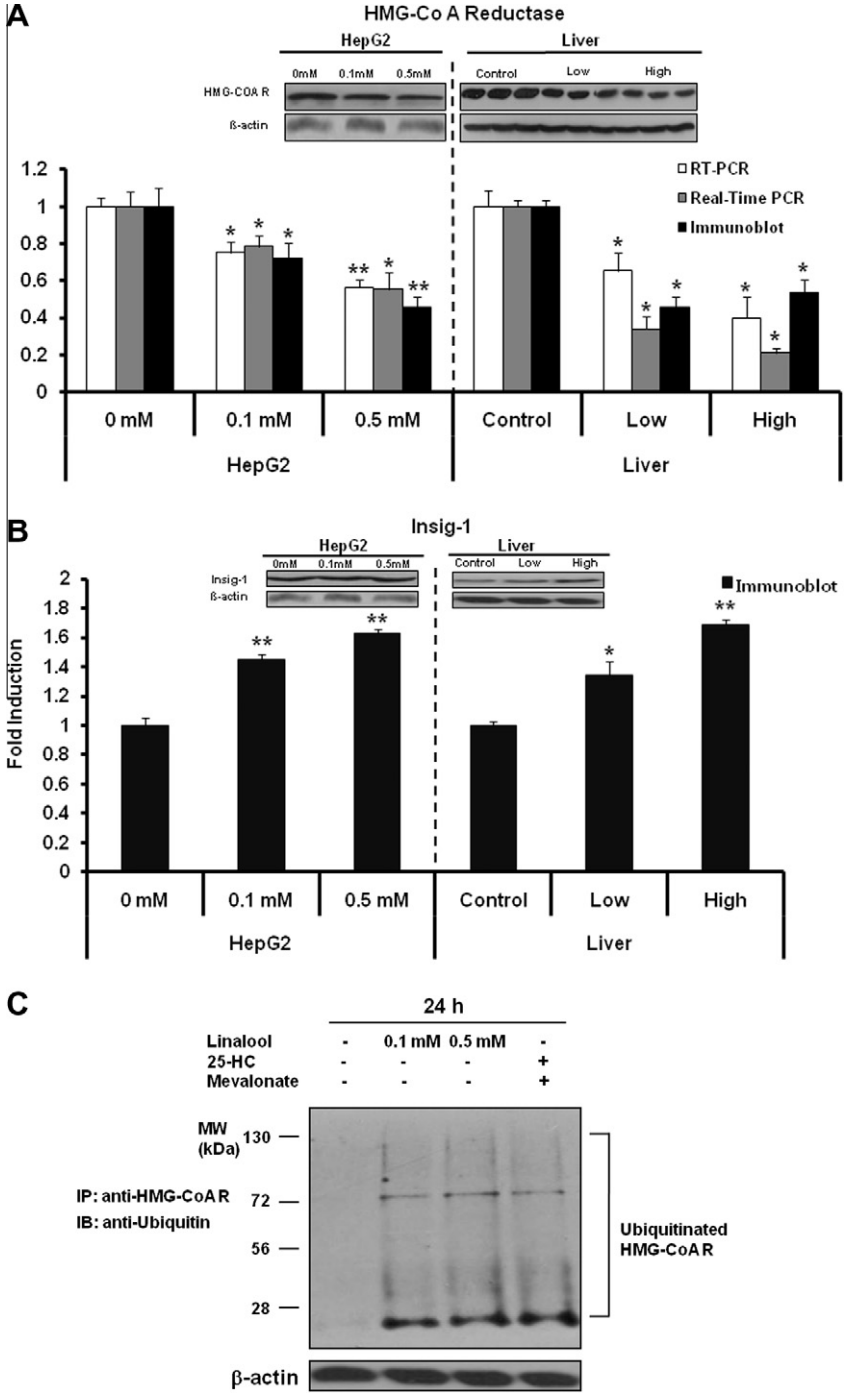

Fig. 3. Expression of HMGCR and Insig-dependent ubiquitination of HMGCR. HepG2 cells and mouse livers after treatment were prepared as described in Section 2. The ubiquitination method is provided in the Supplementary methods. (A) HMGCR mRNA and protein expression. (B) Insig-1 protein expression. (C) Insig-dependent ubiquitination of HMGCR. Cells were treated with 0.1 mM or 0.5 mM linalool, or a mixture of sterols (1 g/ml 25-hydroxycholesterol and 10 g/ml mevalonate) for 24 h at 37 °C. The cells were harvested and subjected to immunoprecipitation with anti-HMGCR IgG and protein A/G Plus agarose beads. Aliquots of cell lysates and immunoprecipitates were subjected to 10% SDS–PAGE, followed by immunoblotting analysis with a polyclonal antibody against ubiquitin (IgG-P4D1; 1:1000). Immunoreactive bands were visualized by ECL with an exposure time of 3–5 min.

with 25-HC and mevalonate for 24 h, ubiquitinated reductase appeared as a high-molecular-weight smear, which was not present in immunoblots of control cells. In the presence of linalool, the cells showed a marked increase in the amount of ubiquitinated reductase (Fig. 3C). These results suggested that linalool reduced the expression of HMGCR via downregulation of SREBP-2 and also promoted the Insig-dependent ubiquitination of endogenous HMGCR protein.

3.4. Binding of SREBP-2 to the HMGCR promoter is reduced with linalool treatment

The HMGCR promoter has multiple transcription factor binding sites: a sterol regulatory element (SRE; −172 to −163) for SREBP-2 and a cAMP responsive element (CRE; −110 to −103) for CREB, which is activated by P300/CBP-associated factor (PCAF) acetylation [18]. This acetylation on the CRE sequence has been shown to induce SREBP-2 binding by stabilizing protein–DNA complexes on the promoter [19]. To investigate the effects of linalool treatment on transcription factor binding to the HMGCR promoter, ChIP analysis was performed with anti-SREBP-2 and anti-PCAF antibodies, followed by real-time PCR analysis using bound and input DNA. In HepG2 cells, both linalool concentrations reduced the binding of SREBP-2 to the HMGCR promoter by up to 70% ($P < 0.05$; Fig. 4B). Similarly, PCAF binding to the CRE was reduced by 57% and 88% at 0.1 and 0.5 mM linalool, respectively ($P < 0.01$; Fig. 4B). These results were confirmed by quantitative PCR analysis, which showed similar trends (Fig. 4B).

4. Discussion

The results of the present study indicated that linalool treatment of HFD-fed mice reduced the total plasma and LDL cholesterol concentrations with a concomitant reduction in hepatic lipid levels. HDL cholesterol levels tended to increase, and this induction could become significant with an increased sample size. In a meta-analysis, a 10 mg/dl reduction in plasma cholesterol levels decreased the CHD mortality by up to 9% [2], which suggests that such a reduction in total cholesterol is possible with linalool consumption from teas and herbs. Therefore, this compound may have significant implications for a reduced rate of CHD in the general population. This possibility should be confirmed in future human studies.

Results showed dose-dependent cholesterol and TG reduction in hepatocytes (Fig. 1B). We used two doses in the present animal study, indicated as low (0.57 mg/mouse/d) and high concentrations (120 mg/mouse/d). Our animal experiment showed that both low and high concentrations of linalool showed similar hypocholesterolemic activities, i.e., a low concentration of linalool is sufficient to reduce plasma cholesterol levels in mice and non-toxic high concentration also showed similar effects. However, both tested concentrations may not have been optimum linalool concentrations for hypocholesterolemic effects, and the results at least confirmed that these two concentrations were effective for reducing total and LDL cholesterol levels in mice. Various concentrations should be tested to determine the optimal dosage for future human trials. Our findings indicated that the hypocholesterolemic effect of linalool was achieved mainly by decreased expression of HMGCR, a marker for hepatic cholesterol synthesis [20]. We showed that the

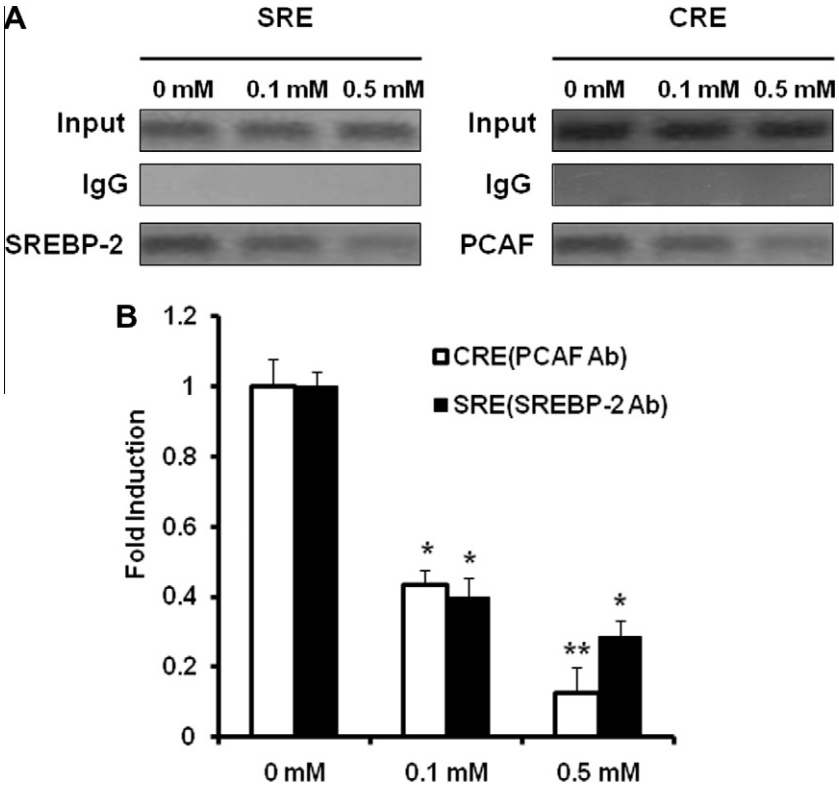

Fig. 4. ChIP analysis of SREBP-2 and PCAF binding to the HMGCR promoter in control and linalool-treated HepG2 cells. The detailed method is provided in the Supplementary methods. Antibodies against SREBP-2 and PCAF were used for ChIP, followed by real-time PCR amplification of the 180-bp region surrounding the SRE and CRE sites of the HMGCR promoter. (A) Immunoprecipitated DNA was amplified with SRE and CRE primers, and the products were resolved in 2% agarose gels stained with ethidium bromide. Input: an aliquot of 10% total DNA input; SREBP-2: samples immunoprecipitated with SREBP-2 antibody; PCAF: samples immunoprecipitated with PCAF antibody; IgG: samples immunoprecipitated with rabbit IgG antibody as control for non-specific immunoprecipitation. (B) Quantification of ChIP samples using real-time PCR. The fold induction was calculated as the ratio between the mean cycle threshold (Ct) value of the input or treated sample and that of the corresponding control. $^{*}P < 0.05$ compared with control; $^{**}P < 0.01$ compared with control.

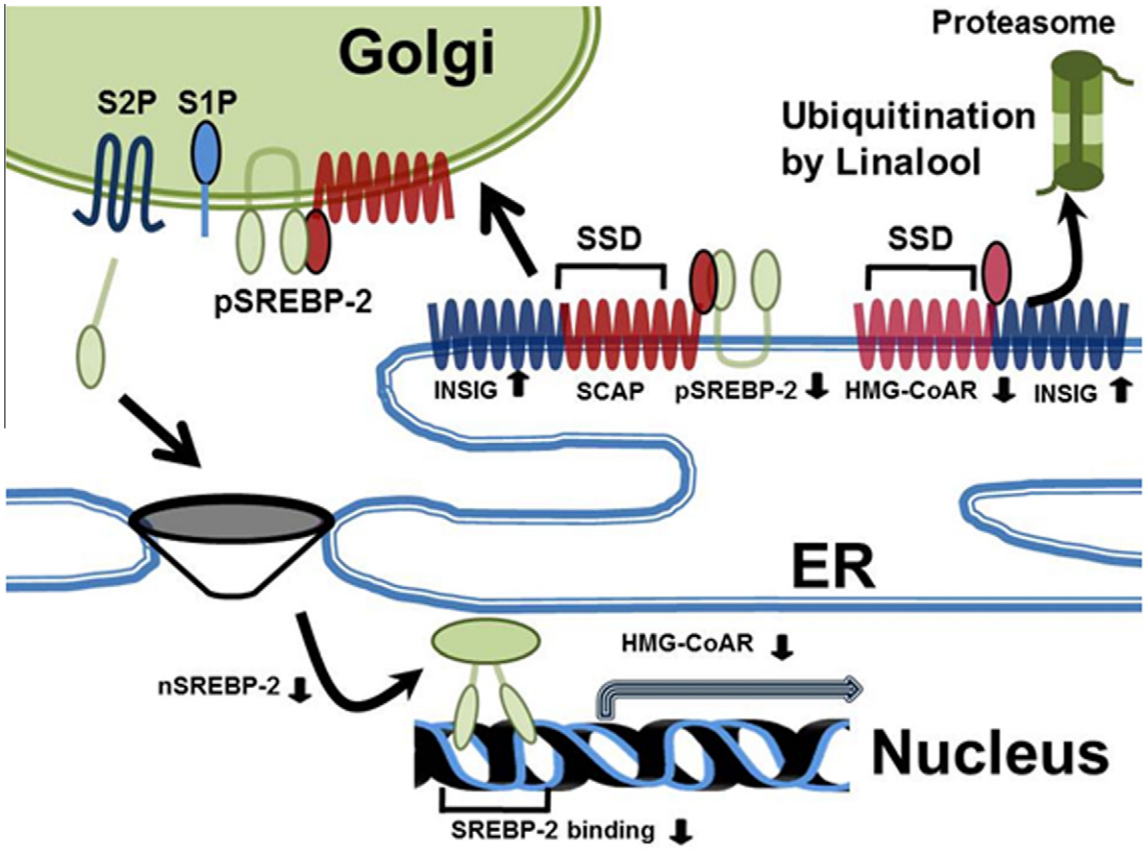

Fig. 5. Proposed mechanisms for the hypocholesterolemic actions of linalool. SSD, sterol sensing domain; SCAP, SREBP cleavage activating protein.

decrease in HMGCR expression resulted in part from a linalool-induced reduction of SREBP-2 expression as well as a linalool-induced acceleration of ubiquitin-dependent HMGCR degradation. The proposed multiple hypocholesterolemic mechanisms of linalool are summarized in Fig. 5.

Several lines of evidence indicated that linalool reduced SREBP-2 expression: qPCR analysis demonstrated a decrease in SREBP-2 mRNA level; immunoblotting, immunohistochemistry, and ChIP analyses revealed reduced SREBP-2 protein levels, especially in its mature nuclear form; and nSREBP-2 decreased its binding to the HMGCR promoter, SRE. The expression of the LDL receptor, a well-known target gene for SREBP-2, was unaltered. The LDL receptor is a well-known target gene for SREBP-2, and its expression is often downregulated with the suppression of SREBP-2 activity in both mice and humans. We do not have a clear explanation for the lack of a change in LDL receptor expression. However, the LDL receptor promoter is operated by multiple transcription factors with both sterol-dependent and non-sterol-dependent mechanisms [21]. Therefore, the possibility exists that the unaltered LDL receptor expression is due to a combined effect of reduced SREBP-2 and non-sterol-dependent mechanisms, which could compensate for cellular cholesterol reduction by HMGCR downregulation.

The SREBP pathway is well understood [22]. The active form of the SREBP transcription factor is released from the amino-terminal domain of the SREBP precursor by proteolytic cleavage in the Golgi apparatus. Two separate site-specific proteolytic cleavages catalyzed by site-1 protease (S1P) and site-2 protease (S2P), respectively, in the Golgi membrane are necessary for the release of the transcriptionally active amino-terminal domain of the SREBP precursor. In addition, the regulated release of transcriptionally active SREBP requires the cholesterol-sensing SREBP chaperone, SCAP, which forms a complex with the SREBP precursor via an interaction between their respective carboxy-terminal domains [23]. The active SREBP is translocated to the nucleus, where it binds to the SRE sequence of target gene promoters to increase transcription rates.

This mechanism is primarily regulated by cellular sterol concentrations. Sterol binding to SCAP under high-sterol conditions allows the reversible binding of SCAP to Insig, another ERresident membrane protein, to form an Insig:SCAP:SREBP triple complex in the ER membrane [24]. Under low-sterol conditions, Insig is released from the complex, and SCAP escorts SREBP from the ER to the Golgi apparatus, where proteolytic cleavage by S1P and S2P release active SREBP from the amino-terminal end of the precursor. After its translocation to the nucleus, SREBP activates HMGCR transcription [25]. In the present study, we showed that linalool administration slightly attenuates nuclear translocation of SREBP-2 by altering the expression of Insig-1. The expression of Insig-1, which enhances ER retention of SREBP-2 [26], was significantly upregulated with linalool. The ratio of the nuclear-to-precursor form of SREBP-2 was somewhat reduced with linalool treatment, and the attenuated nuclear translocation of SREBP-2 could, at least in part, contribute to decreased HMGCR transcription. We also confirmed that linalool directly suppressed the binding of the SREBP-2 coactivator PCAF to the CRE sequence of the HMGCR promoter, suggesting that the reduction of SREBP-2 binding to the HMGCR promoter subsequently decreased the recruitment of PCAF.

HMGCR is also posttranscriptionally regulated by the ubiquitin–proteasome system, which degrades ER membrane proteins through ER-associated degradation [24,27]. The central event in this process is sterol-stimulated binding of the reductase membrane domain to Insig proteins, which are bound to the ubiquitin

ligase, gp78 [28]. The membrane-anchored gp78 mediates ubiquitination of HMGCR, an obligate reaction for accelerated enzyme degradation. Non-sterol, mevalonate-derived products cannot stimulate ubiquitination or degradation of HMGCR in sterol-deprived cells, but can augment enzyme degradation in cells replete with sterols [26,27]. Metabolic evidence suggests that several molecules, including intermediates in sterol biosynthesis, may regulate this process. The 20-carbon isoprenoid geranylgeraniol, a cholesterol biosynthesis intermediate, and lanosterol, a methylated sterol, potently stimulate Insig-dependent ubiquitination and degradation of HMGCR [28]. In addition, oxysterols derived from the conversion of either endogenous or LDL-derived cholesterol also stimulate the acceleration of reductase degradation [29]. Thus, selective recognition of sterol ligands by HMGCR and SCAP appears to contribute to the ability of Insig proteins to mediate the regulation of both proteins through distinct mechanisms. In addition, tocotrienols, naturally occurring forms of vitamin E, are direct, posttranscriptional suppressors that presumably mimic non-sterol isoprenoids in accelerating reductase degradation [24]. In the present study, we showed that linalool mimics non-sterol isoprenoids in promoting Insig-dependent ubiquitination and degradation of HMGCR in addition to reducing SREBP-2 expression. We reasoned that the upregulation of Insig-1 expression with linalool and the two non-sterol isoprene units in the chemical structure of linalool may be associated with the Insig-dependent ubiquitination of HMGCR. Ubiquitin-dependent degradation of HMGCR was markedly induced following linalool treatment. Linalool appears to be less potent than 25-HC, mevalonate, and tocotrienol as high linalool levels (100–500 μM) exerted similar effects to these compounds. Thus, the non-sterol isoprene unit of linalool may exert the effect, which is as effective as that of 25-HC, mevalonate, and tocotrienol.

In conclusion, linalool is hypocholesterolemic with multiple mechanisms of action: decreased mRNA and protein expression of SREBP-2, attenuation of nuclear translocation of SREBP-2 with altered expression of Insig-1, thereby suppressing HMGCR transcription, and finally accelerating the Insig-1-dependent ubiquitination of HMGCR. This is one of few studies describing the molecular mechanism of aromatic food compounds, given that most previous phytochemical studies have investigated lipid-soluble molecules. Our results indicated that food molecules with a pleasant smell may have beneficial metabolic effects with multiple mechanisms of action.

Conflict of interest statement

The authors have declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.09.012.

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